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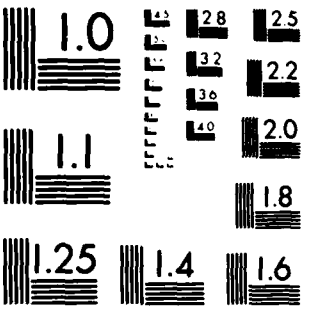

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EVALUATION STUDIES OF THE DEN-2/S-1 VACCINE

Annual Report

Edmundo Kraiselburd, Ph.D.

August 1981

Supported by

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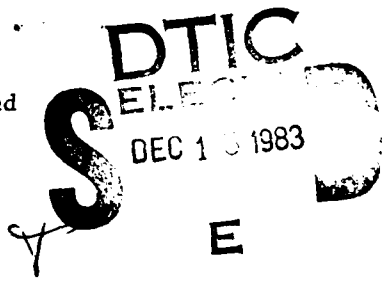
University of Puerto Rico
Medical Sciences Campus, GPO Box 5067
San Juan, Puerto Rico 00936



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Dengue virus immune monkeys

Vaccination DEN-2/S-1 Vaccine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A dengue-2 live virus vaccine was tested in monkeys immune to heterologous dengue serotypes to determine if, as with wild DEN-2 virus, antibody-enhanced viraemia and seroconversion would occur. A low dose of 900 plaque-forming units (PFU) of the DEN-2/S-1 vaccine virus was inoculated subcutaneously into rhesus monkeys six months after they had received wild DEN-1, DEN-2 or DEN-3 viruses, and into non-immune monkeys. As previously reported for non-immune monkeys, there was little, if any, detectable vaccine viraemia in any of the groups of monkeys. There was no difference in seroconversion between the dengue heterologous immune

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20. Abstract (continuation)

(3/6) and non immune (1/3) monkeys. These data indicated that 1) the vaccine virus may differ from the parent virus in the ability to complex with heterologous antibody and thus, in the ability to infect Fc receptor bearing cells in monkeys; (2) 10^3 PFU of vaccine virus is approximately the 50% infectious dose in monkeys as measured by seroconversion.

Kraiselburd, Edmund, Kessler, M., Torres Blasini, G. Lack of Viraemia and Limited Antibody Response of Dengue Virus Immune Rhesus Monkeys After Vaccination With DEN-2/S-1 Vaccine. (Accepted for publication) Transaction of the Royal Society for Tropical Medicine and Hygiene, 1984.

TABLE OF CONTENTS

Accomplishments: A group of 16 tuberculine-negative juvenile rhesus monkeys were pretested for HI and neutralizing antibodies 1,2 and 3 serotypes. All animals were found to be free of dengue virus antibody. All monkeys were housed at the Caribbean Primate Research Center (Sabana Seca) in individual steel squeeze cages in mosquito-proof area, one month before the present bleeding was performed. Three animals were inoculated with D-1, six with D-2 and three with D-3. Four uninoculated monkeys were used as control animals. Dengue virus strains and virus titers used in this experiment are described in Table 1.

Animal inoculation was performed on 3/24/81. Animals were bled daily on post infection days 1,3,5,7 and 9 and undiluted serum samples were assayed for viremia using LLC-MK2 cells. Viremia was detected in all but 3 inoculated animals. As expected no viremia was detected in control animals. Serological tests were performed on sera taken on post infection days 30,56 and 90. HI tests revealed seroconversion in all but one infected animal of the D-2 group (monkey 90A). However, N test revealed that this animal had a titer of 450 on post infection day 30 (see Table 1).

Problems: A N test done with D-1 had to be repeated. This was so because the number of plaques used in the test was 10x lower than expected (due to a mathematical error). This test was repeated using a minimum of 80 plaques for each assay condition and is reported in Table 1.

Since the D-3 virus preparation received from WRAIR had a titer of only 2.1×10^4 pfu/ml, we had to dilute the other virus preparations to obtain comparable titers for animal inoculation. We were told that the WRAIR D-3 virus preparation was accidentallythawed twice (due to a revco failure) before it was sent to our laboratory.

Direct inoculation of sera into Albopictus C6/36 cells was found to be toxic to the mosquito cells. Therefore, indirect viremia assays will be performed with

a 1.3 dilution of sera taken from infected animals.

Future Plans: Future plans call for HI tests on sera taken on day 120, 150 and 180 post inoculation. N tests will be performed on sera taken 150 and 180 days post inoculation. On post infection day 180 all animals (except one control uninfected monkey) will be vaccinated s.c. with a DEN-2/S-1 vaccine preparation (0.5 ml, about 10^5 pfu/ml). Viremia assays will be performed with sera taken on post vaccination days 1 through 10. The size and temperature sensitivity of the plaques obtained in LLC-MK_2 cells will be determined. An indirect plaque assay method will be used in those sera which gave a negative viremia result by the direct plaque assay method. Details of this procedure are described in our contract application.

Plans for year 1982 are described in detail in the enclosed contract renewal application. We have discussed these experiments with Dr. Walter Brandt and we are willing to modify the protocols (included in the contract renewal application) if so requested.

Overtime Work: All personnel involved in this contract worked overtime. This is so because:

1. Serological tests involve much work in tissue culture and most of them were performed on the same day.
2. Viremia studies required working hours during weekends and holidays. In order to handle the administrative aspects of this project, the secretary also had to work overtime. The secretary is resposible for the time consuming processing of each job order and purchase order requisition, for preparing reports of attendance of the personnel involved in this contract in addition to typing, filing and other office work.

In order to make progress in this contract, the PI had no choice but to include

an overtime clause in the contracts signed by the technician, the laboratory assistant and the secretary.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

August 1981
Revised Dec 5, 1983
Date

Edmundo Kraiselburd, Ph.D.
Associate Professor
Principal Investigator

TABLE I

Immune Response of Monkeys to Various Dengue Virus Preparations

Monkey No.	Inoculum	Viremia	HI titer on day				N (t) titer on day		
			Pre	30	56	90	Pre	30	90
B165	D-1	-	*	320	160	160	*	310	158
A396	D-1	+ (day 5)	*	20	80	40	*	*	68
793	D-1	+ (days 3,5)	*	40	40	40	*	*	49
DC5	D-2	+(days 5,7)	*	10	20	10	*	320	**
90A	D-2	+(day 1)	*	*	*	*	*	450	**
865/128	D-2	+(day3)	*	20	20	20	*	840	**
B160	D-2	-	*	10	10	10	*	1,350	**
B5	D-2	+(day7)	*	20	20	10	*	6,000	**
B-3	D-2	+(day 5,7)	*	10	10	10	*	2,560	**
B-465	D-3	-	*	20	40	40	*	1,200	800
B-500	D-3	+(day 5)	*	40	40	40	*	410	250
F3	D-3	+(day 5)	*	20	10	20	*	3,200	540
DD3	control	-	*	*	*	*	*	N.D.	*
EW4	control	-	*	*	*	*	*	N.D.	*
CW9	control	-	*	*	*	*	*	*	*
EB1	control	-	*	*	*	*	*	*	*

* : < 10
**: To Be Done
N.D.: Not Done
- : Negative viremia by direct plaque assay
D-1-2-3: Dengue Viruses type 1,2 and 3 respectively
D-1 virus: Jamaica strain 13802 (MK1, PS1, TRA2) 5-6-80 kindly given to us by CDC (San Juan). It was passaged twice in Albo C6-36 cells in our laboratory before inoculation. Titer on inoculation day: 3.2×10^3 pfu/ml.
D-2 virus: PR 1129 (20% SMB, 1-24-74) given by CDC San Juan. It was passaged twice in Albo C6-36 cells in our laboratory before inoculation. Titer on inoculation day = 7.5×10^3 pfu/ml.
D-3 virus: PR38 kindly given by Dr. Walter Brandt (WRAIR). Titer on inoculation day: 2.1×10^4 pfu/ml.

Monkeys were inoculated with 0.5 ml of the above Dengue Virus preparations and were bled 15 days before inoculation (Pre) and on days 30 and 56 after

inoculation for serology. HI tests were performed by Dr. Gladys Sather, CDC San Juan. N (t) test were performed at UPR Medical School. Monkeys were bled on post infection days 1, 3, 5, 7 and 9 for viremia studies. Monkeys were inoculated on March 24, 1981. HI tests were performed with D-1 (Hawaiian Strain), D-2 (New Guinea C) and D-3 (H87 strain), SLE, YF and WE viruses.

Addendum

Although it was not request by this contract, we have initiated experiments with didemnin A, a depsipeptide from a Caribbean Tunicate. This antiviral drug was kindly sent to us by Dr. Kenneth Rinehart, Jr. (Roger Adams Laboratory, University of Illinois, Urbana, IL 61801). This drug was found to have antiviral activity against herpes simplex viruses (types I and II), vaccinia, influenza PR8, painfluenza 3, coxsackie A-21 and equine rhinovirus (Dr. Rinehart, personal communication). We have found that Didemnin A reduces plaque formation by DEN-1, DEN-3 and herpes simplex virus type 2 (see figures 1 and 2). Fifty percent inhibition of plaque formation by these viruses was observed at drug concentrations (in ug/ml) of 0.14, 0.82 and 2.6 respectively. Drug concentrations above 5 ug/ml were found to be toxic to LLc-MK_2 cells.

Maximum inhibition of dengue-2 virus replication was observed when didemnin A was added to the infected cultures on or before 24 hrs. post adsorption (see Table 2).

Enclosed, please find an abstract sent to the ASTMH Meeting.

P.S.: Results of the experiments performed last year under this contract were sent for publication to Infection and Imnunity Journal. The paper was accepted for publication and will appear in the August issue of the above mentioned journal.

TABLE 2

Effect of the time of addition of Didemnin A (3.8 ug/ml) on Dengue-2 Virus replication.

Treatment	Titer (pfu/ml)	Percent of Con
Control (Normal Media)	598 ± 74	100
Control (1% DMSO)	1,970 ± 253	330 ± 69
Pre-treatment for 24 hrs.	226 ± 39	37.8 ± 13.6
0 (Immediately after adsorption)	15 ± 0	2.50 ± 0.13
24 hrs.	5 ± 0	0.83 ± 0.05
48 hrs.	1,180 ± 153	198 ± 13

1.6×10^6 LLC-MK2 Cells were infected with DEN-2 (PR 1129 P=1 Albo WRAIR) at a moi of 3.9×10^{-3}. At the indicated time intervals, Didemnin A (3.8 ug/ml in Eagles medium containing 20% FCS and 1% DMSO) was added to the infected cultures. After nine days of incubation at 35°C, the cultures were titered on LLC-MK_2 cells and the virus plaques were counted.

Figure 1: Effect of Didemnin A on the plaque forming ability of DEN-1 (Jamaica) and DEN-3 (PR-38) Virus.

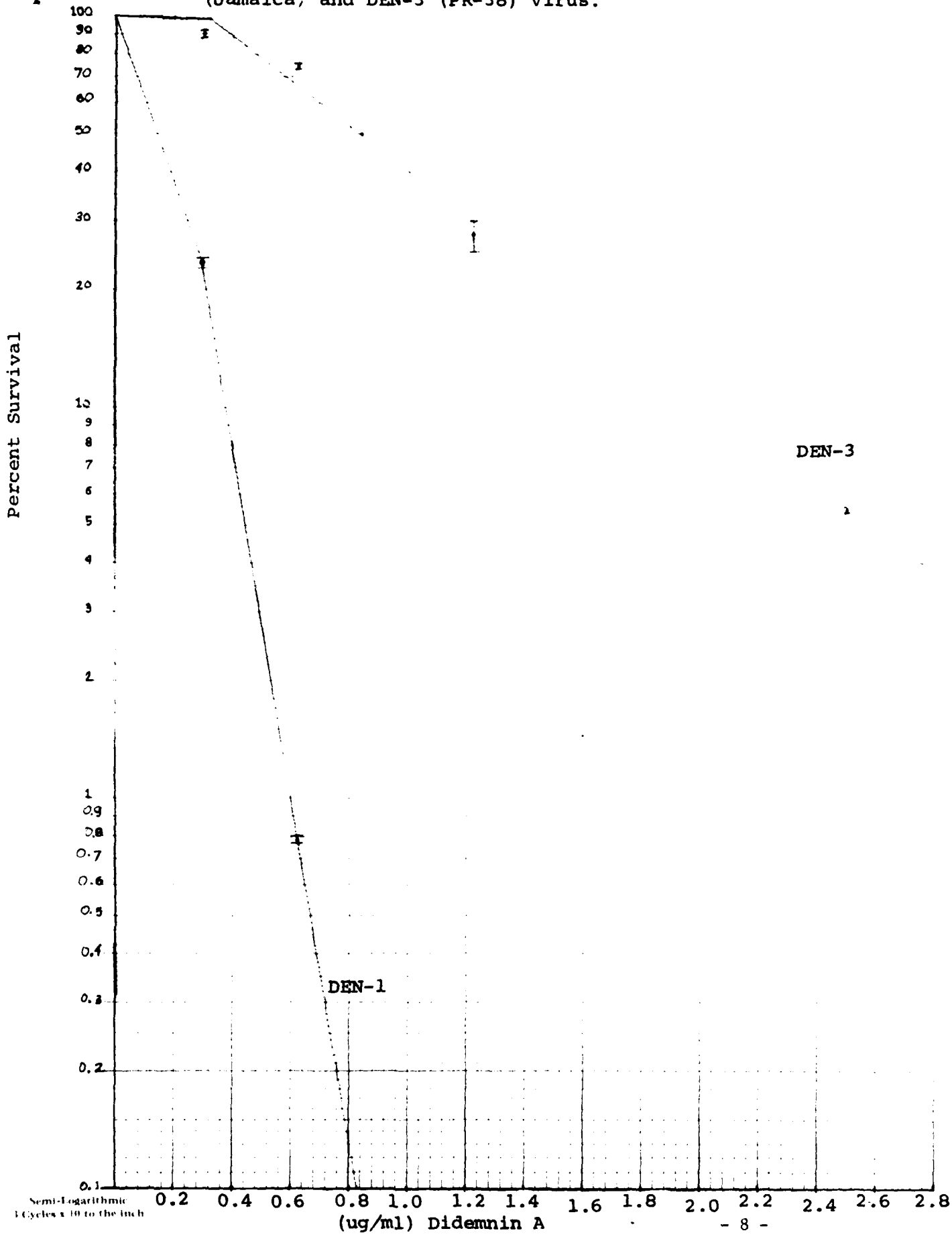
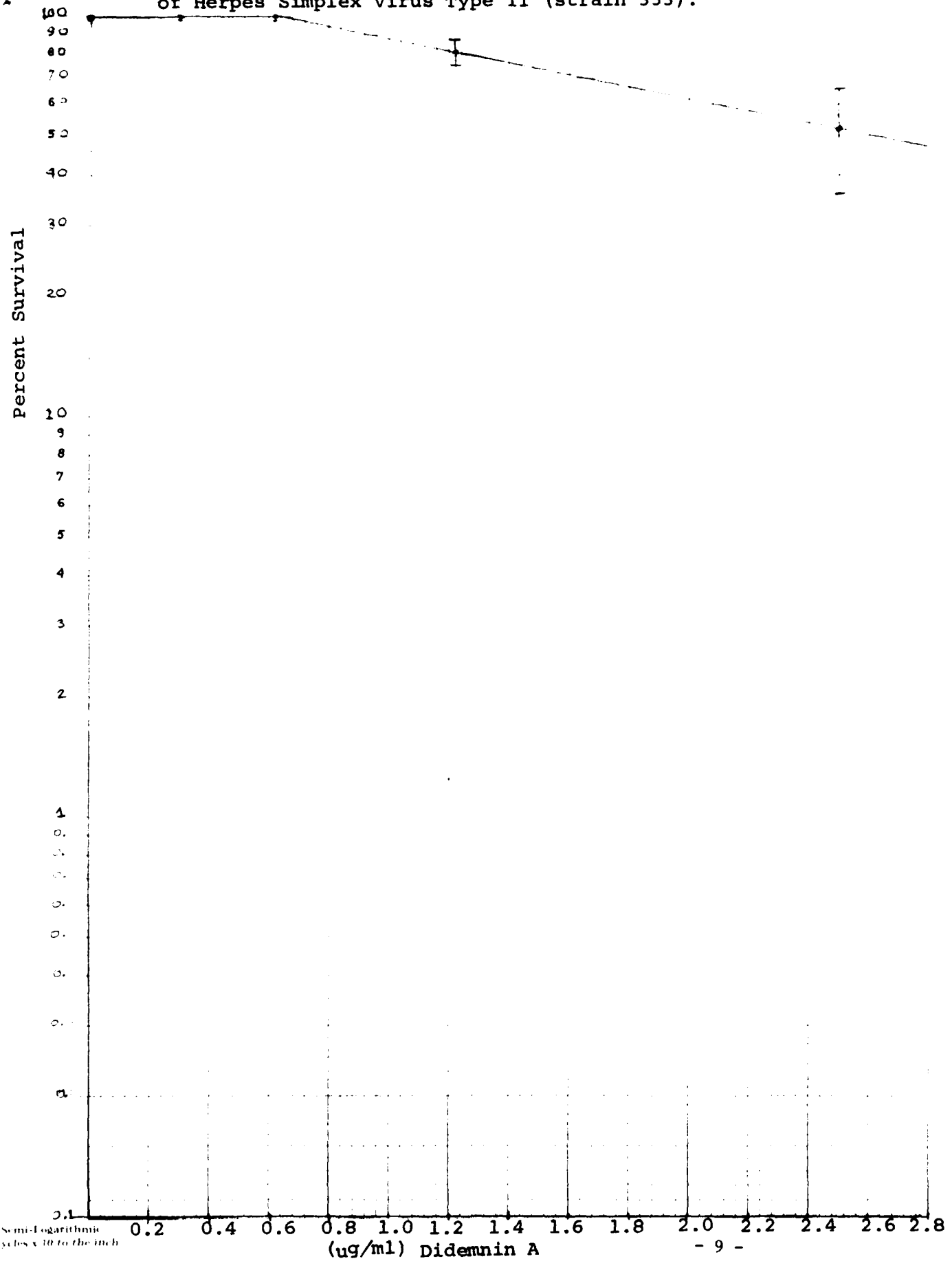


Figure 2: Effect of Didemnin A concentration on the plaque forming ability of Herpes Simplex Virus Type II (strain 333).



Legend to Figures

Figure 1:

Monolayers of LLC-MK_2 cells were infected with either 450 plaques of DEN-1 or 190 plaques of DEN-3. After 1 hr. of adsorption, an agar overlay medium containing different concentrations of didemnin was added in duplicate to the infected cells. After 6 days of incubation at 37°C, the infected cells were stained with neutral red and the plaques were counted. Results are expressed as percent of plaques over the control, untreated sample.

Figure 2:

Wells containing 1 x 10^5 vero cells/ml were infected (in triplicate) with 30 plaques of HSV-2. After 1 hr. of adsorption, different concentrations of Didemnin A were added in F12 medium containing .2% gamma globulin, 2% FCS and 1% DMSO. After 2 days of incubation at 37°C(5% CO_2), cells were stained with cristal violet and plaques were counted.

IN VITRO INHIBITION OF DENGUE VIRUS TYPE-3 REPLICATION BY DIDEMNIN A

Eduardo Maldonado, Julio Lavergne and Edmundo Kraiselburd*. Department of Microbiology and Medical Zoology, University of Puerto Rico, Medical Sciences Campus and Department of Biology, Rio Piedras Campus, Puerto Rico.

The in vitro effect of a new class of depsipeptide, hidroxyisolvaleryl-propionate(didemnin A) (Rinehart K.L., et al., Science 212, 935-937, 1981) on dengue virus Type-3 (D-3) replication was examined. Didemnin A reduced the plaque forming ability of D-3 virus(strain PR-38) on LLC-MK2 cell monolayers. Drug concentrations of 2.1 ug/ml and 0.82 ug/ml reduced the number of D-3 virus plaques by 90 and 50 percent, respectively. No evidence of cytopathic effect was observed at these drug concentrations. However, didemnin A concentrations of 5 ug/ml or higher resulted in marked cell toxicity (See table below).

Preliminary results indicate that antiviral effect of didemnin A was maximal when the drug was added to the infected cultures immediately after virus adsorption. Exposure of the cells to the drug 24 hours before infection did not have any effect on viral replication.

Effect of didemnin A[a,b] on the Plaque forming ability of D-3 Virus

Concentration of Didemnin A(ug/ml)[c]	No. of D-3(PR-38) Plaques	% of Plaques
0 (Control 1% DMSO)	190 ± 4.2	100
5.0[d]	0	0
2.5	10.5 ± 2.1	5.5 ± .8
1.25	53.0 ± 8.4	27.9 ± 3.1
0.62	141.0 ± 2.8	74.2 ± 1.6
0.31	171.5 ± 12.0	90.2 ± 2.4

a. Didemnin A was added to infected cultures immediately after virus adsorption.
b. Values are the mean ± S.E. of the mean from two replica point determinations.
c. Stock solution of didemnin A (10 mg/ml) was made in 100% dimethylsulfoxide (DMSO). Final concentration of DMSO in the agar overlay media was 1% for experimental conditions.
d. This concentration of didemnin A was found to be toxic to cells.

It is concluded that didemnin A significantly inhibits the in vitro replication of dengue-3 virus when used at concentrations that do not produce cellular toxicity.

* Author presenting the paper.

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